Effects of hunger state on flavour pleasantness conditioning at home: flavour-nutrient learning vs. flavour-flavour learning

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Effects of hunger state on flavour pleasantness conditioning at home:

Flavour-Calorie Learning vs. Flavour-Flavour Learning

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Abstract

This study examined acquired liking of flavour preferences through flavour-flavour and flavour-calorie learning under hungry or sated conditions in a naturalistic setting. Each participant consumed one of three versions of a test drink at home either before lunch or after lunch: minimally sweetened (CONTROL: 3% sucrose, 40kcal), artificially sweetened (3% sucrose 40kcal plus artificial sweeteners ASPARTAME) and sucrose-sweetened (SUCROSE: 9.9% sugar, 132kcal). The test drink was an uncarbonated Peach flavoured iced tea served in visually identical drink cans (330ml). Participants preselected as "sweet likers" evaluated the minimally sweetened flavoured drink (conditioned stimulus, CS) in the same state (hungry or sated) in which they consumed the test drink at home. Overall, liking for the CS flavour increased in participants who consumed the SUCROSE drink, however, this increase in liking was significantly larger when tested and trained hungry than sated, consistent with a flavour-nutrient model. Overall increases in pleasantness for the CS flavour in participants who consumed the SUCROSE drink when sated or the ASPARTAME drink independent of hunger state, suggest that flavour-flavour learning also occurred. These results are discussed in light of current learning models of flavour preference.

Keywords: flavour-calorie learning, flavour-flavour learning, post-ingestive effect, nutritional need, home-consumer trial

Introduction

Food preferences are influenced by physiological factors which determine hunger, satiety and satiation, and by a biological learning mechanism which supports the acquisition of food likes and dislikes. Two models implicated in acquisition of flavour liking suggest that flavours can act as conditioned stimuli (CS) which can then be associated with biologically relevant events (unconditioned stimuli: UCS) following the experience of the flavour (Yeomans, 2006; Zellner, 1991). According to the Pavlovian conditioning model, both flavour-calorie learning (FCL) and flavour-nutrient learning are based on associations between the flavour and beneficial or aversive post-ingestive consequences, with the most common beneficial effect being the delivery of energy (see Sclafani, 2001) while flavour-flavour evaluative learning (FFL) is based on associations between a novel neutral flavour (CS) and a second flavour or flavour element which is already liked or disliked (see De Houwer *et al.*, 2001; Yeomans, 2006 for a review).

Of particular interest at present is the role of liking for sweet-flavoured drinks since over-consumption of sugar-sweetened soft drinks has been associated with an unhealthy diet and weight gain (e.g., Alexy et al., 2006; Berkey et al., 2004). However, sweet drinks can either contain energy where the sweetener is a sugar such as sucrose, or be energy free where drinks are artificially sweetened. Liking for the flavour of both sugar-sweetened or artificially sweetened drinks could develop through FFL since in general we express liking for sweet tastes (Steiner *et al.*, 2001), but in addition liking for sugar-sweetened drinks may develop through FCL. Gaining a clearer understanding of how FFL or FCL underlie acquired liking for sweetened drinks is thus important, and was the overall aim of the present study.

The clearest evidence that energy can reinforce changes in flavour preferences comes from animal studies, where neutral flavours are selectively paired with the post-ingestive delivery of energy (see Booth, 1972; Capaldi, 1992; Myers *et al*., 2005; Sclafani, 1999, 2001). In the most sensitive designs, rats are given ad libitum access to two flavoured non-nutritive solutions. Consumption of one solution (the positive flavour cue, or CS+ in Pavlovian conditioning terms) automatically leads to intra-gastric infusion of an energy-bearing liquid. In contrast, consumption of the alternative flavour leads to intra-gastric infusion of water. The outcome of these discrimination learning studies was a profound and durable preference for the CS+ over the CS-. This was true with a large number of energy-containing reinforcers: sucrose (Sclafani, 2002), glucose (Myers & Sclafani, 2001a, b), starch (Elizalde & Sclafani, 1988; Sclafani & Nissenbaum, 1988), fats (Lucas & Sclafani, 1989) and alcohol (Ackroff & Sclafani, 2001; Ackroff & Sclafani, 2002; Ackroff & Sclafani, 2003). Booth (Booth & Davis, 1973; Booth, 1977) reported that rats learned to prefer flavours paired with an energy-rich food when hungry but switched their preference to flavours associated with a low-energy food when sated. These accumulating data strongly suggest that energy is a powerful reinforcer of food preferences in these highly controlled laboratory studies with animals when food deprived. Moreover, in a recent study Yiin and colleagues have suggested that food deprivation enhances the expression but not acquisition of flavour preference conditioning in rats (Yiin et al., 2005).

Despite assertions from animal studies that FCL is probably critical to the development of preference for high caloric foods, specific evidence of equivalent changes in flavour preference in controlled human studies is surprisingly weak, with relatively few studies reporting reliable increases in flavour preference in humans based on associations between flavours and nutrients (Brunstrom, 2005). There is however evidence that children develop a preference for high-energy (carbohydrate) containing drinks relative to low-energy drinks (Birch *et al.*, 1990) and for high-fat paired flavours over low-energy low-fat flavours (Johnson *et al.*, 1991; Kern *et al.*, 1993). In adults, the clearest evidence for nutrient-related preferences were in a study where two distinctly flavoured foods, one low and one high in protein, were consumed after a low-protein breakfast. Under these conditions a preference for the flavour associated with high-protein emerged, with expression of this preference acutely sensitive to the current level of protein deprivation (Gibson *et al.*, 1995). This followed up on an earlier study, where preference for the flavour of a soup with added starch increased relative to a soup with no starch, provided participants were hungry when the soup was consumed (Booth *et al.*, 1982). In a later study, Booth et al. (1994) found that preference for a high-energy novel fruit flavoured yoghurt increased when the hungry-trained group were tested hungry but not when full, with no change in appetite for the low-energy flavour. This increased liking for the high-energy novel yoghurt consumed in a state of high-energy requirement (hunger) has recently been repeated in both laboratory and real world settings (Appleton et al., 2006).

An important element in our understanding of FCL is how acquired liking relates to the current nutritional need of the consumer. The idea that the current physiological needs of consumers for energy (hunger state) influences their hedonic evaluation of a product makes intuitive sense. For instance, acquired liking for a flavour which predicts that a food (or a drink) has a high energy content would be appropriate when the consumer was hungry. For example, Birch and colleagues

(Birch *et al*., 1990) reported children who acquired a preference for a flavour paired with energy when hungry, expressed a lower preference for the energy-paired flavour when sated. Similar state-dependence learning was seen in acquired preference for protein-paired flavours (Gibson et al., 1995). Thus expression of flavour-liking acquired through FCL appears sensitive to hunger-state in humans, and similar findings have been reported in some animal studies. However, it should be noted that not all research findings support state-dependent expression of flavour preferences in FCL. For example, Gibson and Wardle (2001) found that hungry-trained participants increased intake of novel fruit snack bars when tested full, not hungry. The researchers suggested that the hungry-trained group learned that the bars lacked strong satiation, thus allowing greater intake when full. This late-meal preference for low-energy foods has been called "conditioned desatiation" as opposed to learned end-of-meal rejection of rich food termed as "conditioned satiety" (Booth, 1972, 1985).

One of the relevant methodological issues in experimental studies of conditioned flavour preferences is that the taste and palatability of a UCS can be confounded with its post-ingestive (nutrient) properties making it difficult to distinguish between FFL and FCL. Although these learning processes may operate independently of one another (Capaldi, 1992; Myers & Hall, 1998; Warwick & Weingarten, 1994), in a typical ingestive behaviour, both may occur simultaneously. For example, flavour cues present in a food or drink may become associated with other already liked or disliked flavours as well as with the post-ingestive effects of nutrients. One method to overcome this potential problem is to contrast between groups the degree of hedonic change for a flavour (CS+) paired with a sweet and nutritive UCS (e.g., carbohydrate), relative to the same flavour CS+ paired with an artificially sweetened drink that is non-nutritive (e.g., aspartame or saccharin), and a control (CS-) condition with neither sweetness or energy. This approach was adopted here.

This study contrasted acquired liking for flavoured drinks developed either through a combination of FFL and FCL (by increasing sweetness by the addition of sucrose) or FFL alone (by addition of artificial sweeteners). To assess the importance of current nutritional need on acquired liking for the flavours of these drinks, consumers were instructed to drink these products either before lunch when we assumed they were in a state of physiological need for energy (hunger state) and after lunch when we assumed they were not in the same physiological condition (sated state). A common criticism of laboratory-based studies is that they often provide poor models of real-life behaviour (e.g., Meiselman *et al*., 2000), and so allowing drinks to be consumed at home made the study more relevant to real-life experience, in line with a recent study of flavour-caffeine learning in our laboratory (Mobini *et al.*, 2005). Thus the main aim of the study was to examine the acquisition of flavour pleasantness through FFL and FCL under either hunger or sated conditions in a naturalistic setting.

Based on the previous findings reviewed above, we predicted that sated participants would develop similar liking for the flavour of both sucrose-sweetened and artificially sweetened drinks, since both the sucrose and artificially sweetened drinks should support FFL and the energy in sucrose would be less relevant. In contrast, when trained in a hungry state FCL will be more relevant, and so the acquired liking for the sucrose-sweetened drink should be stronger than that for the artificially sweetened drink regardless of hunger-state, or for the sucrose-sweetened drink experienced when sated.

A critical assumption in the present study is that sweet taste alone is sufficient to promote FFL in humans. However, attempts to demonstrate increased liking or preference for flavours and food-related odours paired with sweet tastes have had mixed results, with some studies reporting increased liking (Yeomans *et al.*, 2006; Zellner *et al.*, 1983), but the majority reporting no changes in liking (Baeyens *et al.*, 1990; Stevenson *et al.*, 1998; Stevenson *et al.*, 2000; Stevenson *et al.*, 1995). Recent studies in this laboratory provided insights into the cause of these discrepant results (Yeomans & Mobini, 2006; Yeomans et al., 2006). It is well known that people vary in the degree to which they rate sweet tastes as pleasant, with an apparent distinction between people who can be characterised as sweet likers and sweet dislikers (Looy *et al.*, 1992; Looy & Weingarten, 1991). Studies which failed to find evidence of FFL by pairing sweet tastes with flavours or odours did not ensure that their participants found the sweet taste a pleasant experience. When participants were pre-selected based on screening sweet taste, subsequent experience of flavours with the liked sweet taste reliably resulted in increased flavour-liking through FFL (Yeomans & Mobini, 2006; Yeomans *et al.*, 2006). Therefore, to ensure that FFL reinforced by sweet tastes was possible in the present study, we adopted the same approach of pre-selecting sweet likers.

Methods

Design

Using a between subjects design, we tested the degree to which appetitive state (hungry or sated) affected the acquisition of liking for a novel flavoured drink associated with two sorts of reinforcers: sweet taste or nutritional consequence. Participants consumed one of three versions of a novel-flavoured drink: a sugar-sweetened high-caloric drink, an artificially sweetened low-caloric version or a minimally-sweetened low-caloric drink as control drink. In each of these three conditions, the test drink was consumed either in a hungry (Before-Lunch) or sated (After-Lunch) state giving six conditions in total. Each participant consumed four servings of their assigned drink under naturalistic conditions at home. To assess change in liking for the drink flavour, all participants attended two laboratory-based test sessions, one before (pre-training) and one after (post-training) the home consumption trials, where they made hedonic and sensory evaluations of the minimally-sweetened (CONTROL) flavoured drink. These evaluations were made in the same nutritional need state (hungry or sated) as the home consumption trials.

Participants

A total of 72 volunteers were recruited from a database of students and staff at the University of Sussex who had previously shown an interest in studies relating to eating and drinking. The experiment was advertised as a study of "the perceptual properties of common drinks consumed at home", and respondents were required to attend for an initial screening session to ensure they were sweet likers (details below). Volunteers who were taking medication or had food allergies did not take part in this study. Participants were the first 60 people to meet the study criteria (Mean age=23.52, SD=6.42), 42 women and 18 men. A further 12 people failed the initial screening test. Participants were allocated randomly to one of the six groups. This randomisation produced no significant mismatch in age or gender between groups. However, the number of female participants was higher than males in all six groups. The protocol was approved by the University of Sussex Ethics Committee, and the experiment was conducted according to the ethical standards laid down in the Declaration of Helsinki 1964.

Taste screening.

At the screening session, potential participants evaluated the taste of two samples of 10% sucrose solutions and two samples of still water for pleasantness, sourness, sweetness, bitterness and saltiness attributes using 100-mm line scales end-anchored with “Very unpleasant” scored 0 and “Very pleasant” scored 100 for the rating of flavour pleasantness, and “Not at all” scored 0 and “Extremely” scored 100 for the other taste attribute ratings with the label for the dimension to be evaluated written above the centre of each line. Solutions were presented as 20 ml servings in 50ml glasses, and participants were required to rinse their mouth with water between solutions. In order to ensure that participants were sweet likers, both sucrose solutions had to be rated at least 55pt on the pleasantness scale.

Test drink

The test drink was an uncarbonated Peach flavoured iced tea served in visually identical drink cans (330ml). This drink was selected from a number of fruit drinks evaluated by a group of untrained volunteers (n=10) who did not participate in the main study. The test flavour was chosen since it was rated as neither too unpleasant nor too pleasant (between 40-65pt on 100pt line-scales) and relatively novel flavour (> 55pt). The test drink was produced in three versions: minimally sweetened (CONTROL: 3% sucrose, 40kcal), sucrose-sweetened (SUCROSE: 9.9% sucrose, 132kcal), and artificially sweetened (3% sucrose 40kcal plus artificial sweeteners ASPARTAME). Attempts were made to match this latter drink in sweetness with sucrose-sweetened drink. The closest match was achieved by adding 0.01% aspartame and 0.007% Acesulphame-K to the minimally sweetened drink. Table 1 shows the initial ratings for the test drink. These test drinks were produced specifically for the study by Unilever Research, Colworth, UK.

Procedure

All participants were invited to attend two test sessions, separated by an eight-day period, with testing completed either between 1100h and 1200h for before-lunch groups or between 1330h and 1430h for after-lunch groups depending upon the condition on which the participant had been previously assigned to. The before- and after-lunch test sessions took place in small cubicles in the Ingestive Behaviour Laboratory at the University of Sussex. Participants were instructed to refrain from eating and drinking other than water 1 h before attending these sessions. In after-lunch groups, participants were asked to eat lunch 1 h before the testing sessions.

On arrival at the pre-training on day 1, participants were given a hunger rating sheet and asked to rate their 'hunger', 'fullness' and 'thirsty' states on 100-mm lines scales anchored with "Not at all" scored 0 and "Extremely" scored 100 with the label for the dimension to be evaluated written above the centre of each line. At each testing session, participants were required to provide a saliva sample which they were told would allow verification of the restriction on eating and drinking prior to testing: this was a ruse to ensure compliance and samples were not tested. They were then presented with 50ml of the minimally sweetened (CONTROL) drink and asked to take a sip of the drink and then evaluate it for Novelty, Pleasantness, Sweetness, Sourness, Bitterness using 100-mm line scales end-anchored with “Very unpleasant” scored 0 and “Very pleasant” scored 100 for the rating of pleasantness, and “Not at all” scored 0 and “Extremely” scored 100 for the other attribute ratings with the label for the dimension to be evaluated written above the centre of each line. The same procedure was repeated at the post-training session.

Following completion of the pre-training, participants were provided with four cans of the relevant drink (SUCROSE, ASPARTAME or CONTROL) along with four hunger rating sheets, four tubes for saliva sampling, and also written instructions about how and when they had to consume the drinks at home. They were instructed to keep the drinks in a refrigerator and consume the whole can of the drink at the same time on four non-consecutive days, with the exact time of consumption arranged with each participant to match the study requirements. Participants in the before-lunch groups were instructed to refrain from eating and drinking other than water for the hour before and after consuming the drink, and to consume the drink one hour before they had lunch. Participants in the after-lunch groups were instructed to refrain from drinking other than water for the hour before having lunch, then consume the drink, and refrain from eating and drinking for a further hour. Participants were instructed to rate their hunger state before consumption of each home-consumer drink followed by spitting into the tube for saliva sampling, again to encourage compliance.

Following completion of the four home-consumption trials, participants returned for the post-training evaluation of the test drink. On the completion of post-testing, they were asked an open-ended question concerning the purpose of the study. They then were fully debriefed and paid for their participation in the study.

Data Analysis

Pleasantness and sweetness ratings at the pre- and post-training evaluations were contrasted between groups using 3-way ANOVA, with time of rating (pre or post training) as a within-subjects factor, whether trained hungry or sated and which drink was consumed (SUCROSE, ASPARTAME or CONTROL) as between-subjects factors. Since we predicted significantly greater increases in the pleasantness of the drink trained with ASPARTAME than CONTROL and SUCROSE than CONTROL, planned contrasts were made between these ratings in both the hungry and sated conditions.

Ratings of the sweetness and pleasantness of the two samples of 10% sucrose used in screening were contrasted between groups to ensure the groups were matched in their evaluation of the trained level of sweetness. Participants' hunger ratings were analysed by 3-way ANOVA with the time of rating (pre-training, four training trials and post-training) as a within subjects factor, hunger state (tested before- or after-lunch) and drink to be consumed during training as between-subjects factors. This analysis tested the success of the hunger manipulation and confirmed that drinks were consumed in the correct state during the home consumption trials.

Results

Drink evaluations before and after home consumption

The rated pleasantness of the test drink flavour varied from before to after training depending on which version was consumed during the home consumption phase (Table 2), whether these drinks were consumed before or after lunch and the time of evaluation (pre or post- training: 3-way interaction $F(2,54) = 3.29$, $p<0.05$). In order to identify the nature of this interaction, further analyses treated the data as six independent groups. Analysis of ratings at pre-training revealed no significant differences between groups at baseline, and consequently we calculated changes in pleasantness post-training in order to determine the effects of training on pleasantness evaluations (Figure 1). These changes varied depending on both the trained drink and hunger state $F(2,54) = 3.47$, $p<0.05$.

For participants who had consumed the same minimally-sweetened drink that they were tested with (CONTROL), there was a significant decrease in pleasantness rating under hunger state $t(19) = 2.81$, $p<0.05$. Changes in flavour pleasantness in group APARTAME, who were predicted to increase liking through FFL, were also unaffected by hunger state, $t(19) = 1.56$, NS, and this overall change in pleasantness was significantly greater than in the CONTROL condition $F(1,36) = 9.70$, $p<0.005$. However, in the group who had consumed the drink with added SUCROSE, the change in pleasantness was affected by hunger state $F(1,18) = 5.22$, $p<0.05$, with a larger increase in pleasantness in the group trained and tested before lunch (26 ± 8) than in the group trained and tested sated (10 ± 10). Notably the change in pleasantness for participants trained with ASPARTAME and with SUCROSE after-lunch were similar, but pleasantness increased more in the group trained with SUCROSE before-lunch.

Interpretation of the changes in drink pleasantness post-training relied on the six groups being matched in terms of their liking for sweetness. This was tested using the ratings from the screening session with two samples of 10% sucrose (Table 3). Analysis of variance revealed no significant differences between groups in sucrose pleasantness F=0.30, p=0.91 or sweetness ratings F=0.65, p=0.66, and consistent ratings across the two evaluations.

Analysis of the rated sweetness of the drinks depending on time of rating (pre or post-training), hunger state and training condition revealed no significant interaction effects $F < 1$ (Table 4), however, there was a trend for increased sweetness of the CS in the SUCROSE and ASPARTAME groups, independent of hunger state.

Hunger ratings

Hunger was measured at the pre-training, four home-consumer trials, and post-training sessions in the six groups (Figure 2). ANOVA across the six sessions with hunger condition (before- or after lunch session) and the drink which they were trained with as factors revealed, as expected, a large overall effect of hunger condition $F(1,54) = 74.66$, $p<0.001$, with high ratings before lunch (54.8) and low ratings after-lunch (25.7). These data also confirm that participants complied with the home consumption instructions, since the ratings on training days were made outside of the laboratory, but these ratings did not differ consistently from those at the two laboratory sessions.

Discussion

The main findings of this home-consumer study were: 1) liking for the target drink (CS) flavour tested in a minimally sweetened form (the CS) increased more in the SUCROSE group when trained and tested in a hungry state than when trained and tested sated; 2) liking for the CS flavour increased slightly in participants who had consumed the artificially-sweetened (ASPARTAME) version during training regardless of whether the drink was consumed when hungry or sated; 3) liking for the CS flavour alone decreased when consumed repeatedly suggesting either that the drink had a mildly aversive flavour or that there were monotony effects in that group; 4) there was a trend for increased sweetness for the CS flavour in both SUCROSE and ASPARTAME groups, independent of hunger state.

The findings that liking for the CS flavour increased more when participants consumed the SUCROSE drink in a hungry compared to sated state suggest that this increased liking was due to the nutritional (post-ingestive) effects of the drink. This is consistent with the FCL model which, based on an associative learning explanation, predicts the response to a CS flavour to be altered by association between the flavour and the positive (or negative) consequences of ingestion (Booth, 1985; Zellner, 1991; Capaldi, 1992; Sclafani, 1999). FCL provides an obvious framework through which to explain this acquired liking: the specific flavour of the drink becomes contingently associated with the positive post-ingestive effects of the drink. Some of the clearest evidence for flavour preference based on FCL comes from studies using caffeine as the consequence in human laboratory studies (Richardson *et al.*, 1996; Rogers *et al.*, 1995; Tinley *et al.*, 2004; Yeomans *et al.*, 1998). These caffeine-based FCL studies have shown that moderate caffeine users who consume a novel-flavoured drink with caffeine (CS+) under caffeine deprived condition on several days develop liking for the caffeine-paired flavour (Tinley et al., 2004; Yeomans *et al.*, 2000; Yeomans *et al.*, 2001; Yeomans *et al*., 1998). More recently, we found that flavour liking conditioned by post-ingestive effect of caffeine can be found under more naturalistic conditions than in the laboratory (Mobini *et al.*, 2005). Consistent with these findings, most recently Appleton et al. (2006) have found that likings for flavours can be conditioned in the real world as part of every day life as well as in the laboratory. Thus both caffeine-reinforced and energy-reinforced FCL can be acquired under naturalistic conditions, adding to the evidence suggesting FCL plays an important role in flavour-preference development.

The slight increased pleasantness of the CS flavour in participants who consumed the artificially-sweetened (ASPARTAME) version both when sated and hungry, and the SUCROSE sweetened drink when sated, can be interpreted in terms of FFL reinforced by drink sweetness. Previously, evidence for increased flavour-liking for FFL reinforced by sweetness in humans has been patchy, as discussed in the introduction. The present data provide further evidence that FFL can occur with sweet tastes for people who express a liking for sweet likers. These data are also consistent with animal studies suggesting that flavour-preferences acquired through FFL are not modified by hunger state (Capaldi *et al.*, 1994; Fedorchak & Bolles, 1987). However, a recent study in our laboratory found evidence that expression of acquired liking for a flavour acquired through odour-sweetness associations was sensitive to hunger state (Yeomans & Mobini, 2006). The difference between that and the present study was that here participants were always tested in the trained state, whereas in Yeomans and Mobini (2006), participants were trained hungry and tested hungry or sated. Thus although the current study found equivalent increases in liking for the flavour CS paired with ASPARTAME when trained and tested hungry or sated, the expression of this acquired liking may vary if tested in a different state to that used in training.

Furthermore, the decreased liking for the CS drink in the CONTROL group can be explained based on FFL. As the participants were sweet likers based on their ratings of 10% sucrose, exposure to 3% sucrose in the CS flavour may have been an aversive experience for these sweet likers, consequently resulting in the development of a conditioned dislike for the control drink flavour.

In summary, the findings of this study suggest that both FCL and FFL processes are important for flavour preference development with preference acquisition through FCL dependent on hunger state. The study also demonstrated that these acquired preferences occur under naturalistic conditions.

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